



## **Hyper- and hypo-biofilm forming mutants of *Listeria monocytogenes* G (Serotype 4b)**

Huang, Yanyan; Zhu, X.; Shi, X.; Knøchel, Susanne

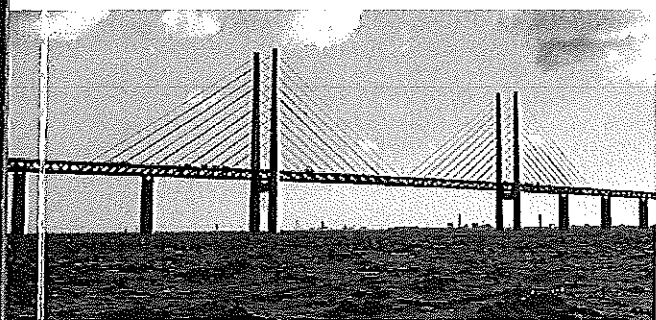
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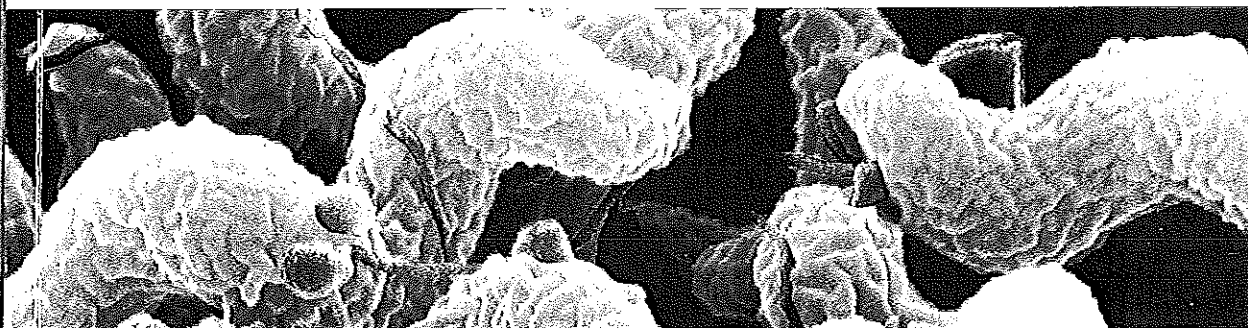
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jaard	PED2.04	Kanno S	PEB1.13	Kocevski Dragana	PED2.53	La Storia, A	PSA2.04
	PED2.01	Kantikova M	PEA1.78	Kocharunchitt Chawalit	PEB2.53	Labbe A	PED1.07
	PED1.33	Kapetanakou A	PEB2.50	Koike ST	PED1.10		PED1.08
	PED1.23		PEC2.46	Komura Tomomi	PEE2.07		PED1.22
	PEE2.22	Kapetanakou, Anastasia	PSB1.06	Kondili A	PEC1.96	Lacour, B	PSD2.04
	PEB2.45	Karamad Dina	PEA1.64	Konrad R	PEE2.11	Lacroix C	PEA1.23
	PSE1.02	Karbancýglu-Guler F	PED1.32	Koo MS	PED1.34		PEA1.25
	PED1.34	Karbancýglu-Güler Funda	PEB1.15	Kopečný J	PEE2.23		PEA2.25
	PED2.52	Karbassi A	PEA1.64	Korenova J	PEB2.32		PEA2.26
	PEA1.36	Karlsen H	PEA1.57	Korkeala H	PEB2.11		PSE1.01
	PEA1.37		PED1.31		PEB2.13	Laghi L	PEA1.69
	PEA1.40	Karpiskova R	PEA1.09		PEB2.14	Laghi L	PEB2.67
	PEA1.41		PEB1.05		PEB2.54	Laghi, L	PSA2.04, PSE1.05, PSE1.06
	PEB1.32		PEC1.10		PEB2.60	Laht T-M	PEA1.15
	PEE2.15	Kashi Y	PEE2.04		PEC2.52	Lahti E	PEC1.92
ien	PEE2.24	Kashi Yechezkel	PEA1.67	Korkeala, H	PSB2.01, PSD1.02	Lahti, E	PSD1.06
	PEA1.10		PEA1.66	Korošec Ž	PED2.52	Lahtinen S	PEE2.24
	PSD1.03		PSE2.03,	Kostelanska M	PEA2.10	Laine P	PEA2.28
	PEE2.21	Kasimoglu Dogru A	PEB1.01	Kostic Tanja	PEC1.99	Lamalian J	PEA1.64
	PEE2.20	Katz T	PEA1.66	Kostrzewa Markus	PEA2.29	Lamberti C	PEA2.36
	PED1.34	Katz T	PEA1.67	Kot, WP	PSD2.03	Lamprecht C	PEC1.18
	PEB2.40	Keller D	PEB1.24	Kouete Kongni V	PED1.28	Lanciotti R	PEA2.43
	PEC1.56	Kentish S	PEE2.02	Koutsoumanis Kostas	PEC1.43		PEB2.67
	PEC1.86	Khamisse Elissa	PEB2.49	Koutsoumanis KP	PEA2.24		PED2.17
	PEB1.26	Khan Nazer AH	PEA1.02		PEB2.41		PED2.58
	er BGM	Khen B	PEC2.58		PSA2.05,		PEE2.08
	PEA1.55		PEC2.59	Kovač K	PEC2.32	Landgraf M	PEC2.22
	PEB2.57	Killer J	PEE2.23	Kowalczyk Magdalena	PEA1.46	Laniewska L	PEB2.24
	PEB2.16	Kim D-H	PEC1.39	Kowalik J	PEC1.28	Laroche M	PED1.04
	PEC2.06	KIM H-n	PEE2.20	Kowalik Jaroslaw	PEC1.27	Larsen M	PEB1.21
r BGM	PED1.29	Kim H-n	PEE2.21	Kozlinskis Emils	PEA1.48	Larsen Marianne H	PEB1.23
	PED1.37	Kim H-Y	PEC2.03	Kriščiunaite T	PEC1.24	Larsen Nadja	PEE2.14
	PEC2.01	Kim Hyun Jung	PED1.34	Kristek S	PED2.25	Larsen, Nadja	PSE1.03
	PEC1.11	Kim Y	PED1.34		PED2.53	Larsson J	PEC1.11
	PSC1.06,	Kim YG	PEC1.42	Kristensen NB	PEA2.44	Lassen J	PEC2.08
	PSA1.04	Kim Y-G	PEC1.39	Kron Morelli R	PEA1.29	Laukkanen, R	PSD1.02
	PEB2.10	Kim Yungyeong	PEC1.38	Kuchta Tomas	PEA1.05	Lavaud A	PEC1.09
	PEA1.61	Kinètiè A	PED2.52	Kuchta Tomas	PEB2.32	Lazzi C	PEA2.09
	PEC2.52	Kirezlieva K	PEC2.34	Kudirkiene Eglė	PEB2.38		PEE2.09
	PEC1.24	Kirilov N	PEA2.17	Kuijper E	PEC2.05	Le Bihan Y	PEC1.31
	PEA2.12	Kita T	PEB1.02	Kumar, Rajesh	PSA2.02	Le Bivic, P	PSA1.06
	PED2.05	Kjeldgaard Jette	PEC1.68	Kunkulberga D	PEA1.48	Le Doeuff C	PEC2.44
	PEA2.19	Klanènik A	PED2.52	Kwak H	PEC1.38	Le Gall, G	PSC1.02
	PEA1.47	Klinder A	PEE1.02	Kwak HS	PEC1.42	Le Marc Y	PEC1.49
	PEA1.52		PEE2.25	Kwon JH	PEB2.10	Lebeau, B	PSC2.03
r BGM	PEA1.60	Klinder Annett	PEE2.26	Kwon YK	PEB2.10	Lebecque A	PEA2.04
	PEB2.33	Klinder, A	PSE2.05	Kümmel J	PEC1.95	LEE H-J	PEE2.20
	PEA2.33	Knauder E	PED2.52	Kümmel J	PEC1.98	Lee H-J	PEE2.21
	PEB1.02	Kneifel W	PEC1.88	Kütt Mary-Liis	PEB1.03	LEE J-e	PEE2.20
	PEB1.13	Kneifel W	PEC1.89	König M	PED1.15	Lee J-e	PEE2.21
	PEC1.26	Knockaert D	PEA2.47	La Gioia F	PEA1.30	Lee J-W	PEC2.03
	PEA1.67	Knudsen, GM	PSC1.04	La Storia A	PED2.20	Lee N	PED1.34
	PEB2.10	*Knøchel S	PEB2.36		PED2.31	Leguerinel I	PEB2.15
	PEB1.26		PED2.10	La Storia Antonietta	PEA2.15	Leguerinel, I	PSC1.01
	PSC1.01	Kocevski D	PED2.25		PED2.23	Lehner D	PEC1.89

el plates with grooves

icult to clean and disinfect. On  
uding *Listeria* spp. In this study,  
of 0.5, 0.2, 1, 2 or 5 mm. The  
exposed to chlorine (Suma Tab  
nonium compound, 740 mg/l)  
pensions of milk, ham, smoked  
and dry (Rh=50%) conditions.  
old, mixed biofilms at 15°C, in

ed with Qac, while the use of  
300 mg/L gave reductions of  
nd 2-4 log units for chlorine  
og factor 2. The reduction of  
reductions were obtained (4  
ly). The reduction of the test  
s, and in the shallow grooves  
with the same concentrations  
re not resistant. Probably the  
ize accumulation of *Listeria*  
ment, incorporated with ef-

and eating food conta-  
occur through contacting  
mperfect and/or irregular  
ome unfavourable con-  
ates, industry isolates,  
to grow and form bio-  
water with 0.05 % or 1  
ystal violet. *LuxS*, gene  
m formation process,  
mplementary to the *luxS*  
xS by polymerase chain  
A subunit of topoisome-  
ernalin protein), provi-  
mplicon needed 50 cycles  
ained in all strains - in  
nt 3 or 4 fragment sets,  
nined strains different  
t sequence. Two *luxS*  
conditions.

#### \* PEB2.36 Hyper- and hypo-biofilm forming mutants of *Listeria monocytogenes* G (Serotype 4b)

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(2) Joint Sino-US Food Safety Research Center and Bor Luh Food Safety Center, School of Agriculture Et Biol-  
ogy, Shanghai Jiao Tong University, PR China

The foodborne pathogen *Listeria monocytogenes* is a serious public health concern. The serotype 4b strains account for about 40% of the sporadic cases and the majority of the outbreaks. Some *Listeria monocytogenes* form biofilm and this is a cause of concern for the food industries. This study was undertaken in order to improve our understanding of the biofilm formation by identifying genes associated with hyper- or hypoformation. **Methods:** A library of more than 2000 Tn917 insertion mutants was constructed by insertion of transposon Tn917 in *L. monocytogenes* G (serotype 4b) chromosome DNA. Biofilm-formation mutants were identified by use of a microtiter assay employing 1% crystal violet. The insertion sites of Tn917 were found by inverse PCR and BLAST analysis, and the genes responsible for changes in biofilm-formation were identified. Those genes were knocked out by homologous recombination to study the function and mechanism in biofilm formation using proteomics technique. **Results:** After screening a subset of the library 1 positive and 10 negative biofilm-formation mutants were found. Four genes were identified. One associated with hyperproduction Im.G\_1771, and three with decreased production: *gltB* (Im.G\_1758); Im.G\_1497; Im.G\_2324, encoding an ABC transporter-permease, glutamate synthase, a MerR family transcriptional regulator, and a conserved hypothetical protein, respectively. After further studies, we found that the *dlt* operon encoding the enzymes incorporating D-ala residues into lipoteichoic acids (LTAs) was strongly down-regulated in the positive mutant. Moreover, the mutant was found to be more sensitive to some membrane active antimicrobials after using a standardized MICs microdilution assay. One of the biofilm negative mutants, the *gltB* insertion mutant, was found to be sensitive to oxidative stress and the inactivation of its upstream gene which encodes a LysR family transcriptional regulator could also cause a decrease in biofilm formation.

#### PEB2.37 Response to abiotic stresses in *Oenococcus oeni* PSU-1: a whole-transcriptome view

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*M Pezzotti* (1), *S Torriani* (1)

(1) University of Verona, Italy

*Oenococcus oeni* is the most important bacterial species for winemaking, as strains of this taxon are the principal actors of malolactic fermentation (MLF). MLF follows alcoholic fermentation and is highly desired in certain styles of wines, both white and red, as it confers positive characteristics in the sensory quality and improves their microbial stability. Scarceness of nutrients, acidic pH, high ethanol content and other stress factors make wine a hostile environment for bacterial growth. However, *O. oeni* is a lactic acid bacterium well adapted to this ecological niche and shows several genomic peculiarities, e.g. the lack of the mismatch repair (MMR) system. Those traits make it a very important bacterium not only for applied purposes but also for basic research, and it could be considered a model organism for Gram-positive bacteria besides *B. subtilis* and *L. lactis*.

In the present study a whole transcriptome analysis was performed on the *O. oeni* model strain PSU-1 by using a custom 12k Combimatrix chip with 1741 probes specific for almost all the ORFs and pseudogenes annotated for the strain (<http://ddlab.sci.univr.it/FunctionalGenomics/datasheets/Ooeni1.0.html>).

The experimental design was aimed at evaluating differential gene expression in comparison to laboratory conditions for optimal growth. Shock conditions tested were presence of 10% v/v ethanol, pH 3.5, and both conditions applied simultaneously, besides heat shock at 42°C and shocks were applied for 6 hours to a mid-exponential grown culture.

Results showed that the major modifications of gene expression were obtained when heat shock and the combination of pH and ethanol were applied, while pH variation appeared to be the most tolerated condition. Interestingly, pseudogenes were expressed in almost all conditions, confirming the suggested hypothesis that they still belong to the stress response machinery of the cell and that they could be functional in other *O. oeni* strains. More in general, the results of this study will have an impact on the comprehension of the general mechanism of stress response in *O. oeni* compared to the model organisms of Gram-positive bacteria, and also in giving useful information to improve procedures of biomass preparation and management of MLF in wine.